ability to measure such fields would help to elucidate the importance of ''electrostatic structure'' to a biosystem's function. Our approach of measuring internal electric fields relies on changes to electronic transition energies of a chromophore when exposed to an electric field, i.e., the ''Stark effect''. Analyzing the Stark shifts allows for extraction of magnitude and orientation the internal electric field. Classically, the Stark shift is analyzed with a series of terms that are linear, quadratic, etc., in the electric field, which requires knowledge of the polarizability tensors for both ground and excited states to extract the internal electric field. Usually the analysis is restricted to term linear in the external electric field, which for randomly oriented chromophores leads to a systematic error. We pose a quantum-mechanical alternative to the classical analysis based on infinite-order perturbation theory that overcomes the neglect of non-linear terms. The challenge with the quantum-mechanical analysis then becomes that, theoretically, it may require the description of an infinite number of electronic transitions to account for the Stark shift. Here, we present a method for determining the number and identity of electronic transitions that are required for quantum-mechanical Stark analysis, essentially opening a practical path to using this more exact analytical option. As demonstration, we apply this new protocol to a porphyrin sensor used to measure the internal electric field of myoglobin and show the accessibility of the method with reasonable computational resources.

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MD + QM Investigations of the Length Scale and Forcefield Dependence of the Time Dependent Fluorescent Stokes Shift of Wild Type Staphylococcal Nuclease and Charge Mutants

J. Nathan Scott[1], Patrik R. Callis[2].
[1]Chemistry, Saint Francis University, Loretto, PA, USA, [2]Chemistry and Biochemistry, Montana State University, Bozeman, MT, USA.

Discussion persists as to the origin of the time dependent fluorescence spectral (Stokes) shift (TDFSS) in the range 100 fs to more than 100 ps for a number of tryptophan (Trp)-containing proteins. TDFSS reports on the dynamic reorganization of the local environment around the large dipole of Trp following excitation to the ^{1}La state. Much of the discussion centers on the ubiquitous ''slow'' (10 ps-5 ns) TDFSS component found only in proteins. Details of what determines the fast (<2 ps) component in proteins are also of interest. Interest focuses on two questions: (1) what are the relative contributions of protein and water; and (2) what length scales characterize these contributions? We have published extensive equilibrium MD+QM simulations on the single Trp of wild type Staph. nuclease, and four charge-changing mutants, all of which experimentally exhibit longer-decaying TDFSS (70-150 ps) than in other proteins, e.g., monellin and GB1 [D.P. Zhong, S.K. Pal, A.H. Zewail, Chem. Phys,Lett. 503 (2011), p. 1-11.] In this work, we have performed non-equilibrium, direct relaxation MD+QM molecular dynamics simulations for the wild type Staph. nuclease to investigate the origin of these long decay times, using both OPLS /TIP3P water and AMBER99sb-ildn/TIP4P water force fields. Initial results suggest that while OPLS fails to capture these longer relaxation times, AMBER99sb-ildn shows much closer agreement with experiment. We will also report results for the four charge-changing mutants and comparisons with other forcefields.

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Detecting Counterfeit Pharmaceuticals through UV Spectrophotometry

Gabriela Figueroa[1], Luis A. Palacio[2], Bruce D. Ray[2], Horia I. Petrache[1], Alfredo Lopez-Yunez[3].
[1]Department of Biology, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA, [2]Physics Department, Indiana University-Purdue University Indianapolis, Indianapolis, IN, USA, [3]Alivio Medical Center, Indianapolis, IN, USA.

Abstract: According to the World Health Organization between 10%-30% of medicines, in Africa, Asia and South America, are counterfeit or substandard, affecting the health of millions of people. Currently, there is no effective way to check the quality of a medicine at the point of care, leaving many with treatable diseases at risk. The goal of this study is to identify UV-Vis (240nm - 500nm) absorbance patterns that would indicate if a drug is substandard or counterfeit. UV-Vis spectroscopy was selected as the method for testing due to the maturity and availability of the technology. Pure Acetaminophen and Tylenol were used as controls for proof of concept. Samples were prepared by dissolving different combinations of the pure active ingredient and adulterants such as cement, rice flour, vitamin C and lactose in three different types of solvents (H2O, 0.1 M HCl, 0.1 NaOH). Various concentrations (ranging from 0.01mg/ml to 0.04mg/ml) and mixing ratios were analyzed using a UV-Vis Spectrophotometer. It was found that adulterants significantly decrease the absorption of acetaminophen at 245nm by interacting with its benzene ring, while showing a slight increase in other parts of the spectrum. UV-Vis scans show that the amount of change in absorbance at specific wavelengths, coupled with characteristic wavelength shifts produced by different solvents, can be used for detection of counterfeit drugs. The methods presented here could be used for quality control of medicines at or near the point of care in parts of the world at higher risk of encountering defective pharmaceuticals.

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Hyperspectral Analysis of Laurdan Emission Spectra in Red Blood Cells and Giant Unilamellar Vesicles

Catherine Leonard[1], Abdelmounaim Errachid[2], Julie Daubie[2], Didier Beghuin[3], Pierre-Jacques Courtois[2], Marie-Paule Mingeot-Leclercq[1], Donatienne Tyteca[2].
[1]Louvain Drug Research Institute (LDRI) - Cellular and Molecular Pharmacology Unit (FACM), Université Catholique de Louvain (UCL), Brussels, Belgium, [2]de Duve Institute (DDUV) - Cell Biology Unit (CELL), Université Catholique de Louvain (UCL), Brussels, Belgium, [3]Lambda-X, Gosselies, Belgium.

Lipid rafts are membrane microdomains enriched in cholesterol and sphingolipids that regulate the activity of several membrane proteins and their associated pathways [1]. Laurdan, a fluorescent probe exhibiting a blue shift in emission (from 440 to 490 nm) upon decreasing membrane order, has been extensively used to quantify and visualize membrane domains [2]. We here applied hyperspectral microscopy to gain better insights on the variation of Laurdan emission spectra in relation with membrane composition. As models, we used red blood cells (RBCs) as the simplest and most characterized cell system, and giant unilamellar vesicles (GUVs) of defined compositions (dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC) and DOPC/DPPC/Cholesterol 1:1:1).

In preliminary studies, we validated an hyperspectral microscope prototype for Laurdan imaging on GUVs and RBCs by comparison with spectrofluorimetry and biphoton confocal microscopy for which reports are available [3]. This microscope yielded better spectral resolution (steps of 1 nm) as compared to confocal microscopy (steps of 10 nm). This improved spectral resolution detected, besides well-known peaks at 440 and 490 nm, new peaks in the Laurdan spectra of GUV (made of DOPC/DPPC/Cholesterol 1:1:1) and RBC membranes around 470 nm (and 540 nm). The biological significance of these new peaks needs to be identified and will be discuss according to experiments performed on GUV displaying different order and composition properties (gel (DOPC), liquid-disordered (DPPC) and liquid-ordered (DOPC/DPPC/Cholesterol 1:1:1) phases).

To conclude, hyperspectral microscopy opens new perspectives for the use of environment-sensitive fluorescence probes, thus new possibilities of understanding membrane domains behavior.

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3138-Pos Board B568

Detection of Interactions of Inositol Phospholipids with Ion Channels

Felix Chin[1], Anoop Saxena[2], Feng Qin[3], Ping-Chin Cheng[3].
[1]William East High School, East Amherst, NY, USA, [2]SUNY Buffalo, Buffalo, NY, USA, [3]SUNY-Buffalo, Buffalo, NY, USA.

Inositol phospholipids account for only a small percentage of total lipids in cell membranes. But they play crucial roles in diverse cellular processes such as signal transduction, cytoskeleton organization, membrane trafficking and membrane permeability and transport. While some of the functions involve regulated hydrolysis to generate second messengers, others are thought to involve direct interactions with proteins. The repertoire of phosphoinositide-binding proteins has been dramatically expanded in recent years. These include many ion channels, from the first identified prototype inward rectifier potassium channel (Kir) to the more recent transient receptor potential channels. However, in most cases the roles of the lipids are implicated from pharmacological maneuvering of signaling pathways. A direct observation of the interaction between lipids and proteins has rarely been possible. The recognition sites on the proteins are often interfered by structural-functional studies using site-directed mutagenesis. Owing to the complexity of gating mechanisms, the molecule sites identified in this manner have been less reliable. In this work we present a FRET approach for direct detection of interactions between phosphoinositide lipids and ion channel proteins. Using an inward rectifier potassium channel and a transient receptor potential channel as prototype models, we show the feasibility to generate recombinant proteins incorporating genetic donors. We use fluorescent lipids as acceptors and show that the energy transfer is limited to only peripheral lipids around channel proteins. Our experiments demonstrate that the assay has a resolution to probe specifically bound lipid molecules.